EFFECTS OF ACUTE AND SUBACUTE CANNABIDIOL TREATMENT ON HEPATIC DRUG METABOLISM

by

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ABSTRACT

Cannabidiol (CBD) was administered both acutely and subacutely to mice, and the resulting effects on hexobarbital sleep time and on the hepatic microsomal drug-metabolizing system were assessed. Acutely administered CBD significantly prolonged hexobarbital sleep time, an effect that persisted for as long as 24 hr; but following subacute treatment, tolerance rapidly developed to the CBD effect. Brain hexobarbital concentration upon awakening was unchanged by either acute or subacute CBD treatment, which suggests that neither the acute effect of CBD on sleep time nor the tolerance that develops in subacutely treated animals is the result of a central interaction of the drug with the barbiturate. Acute CBD treatment increased the half-time of hexobarbital in the brain, evidence that the drug decreased the rate of hexobarbital metabolism. Tolerance was accompanied by a decrease in the elevated half-time of brain hexobarbital, suggesting that tolerance was a consequence of a return toward the normal rate of hexobarbital metabolism. Acutely administered CBD caused a 30 per cent depression in cytochrome P-450 concentration; the duration of the decline approximated

the duration of CBD's prolongation effect on sleep time. Following subacute treatment, cytochrome P-450, total liver protein and microsomal protein concentrations were the same as the controls, but NADPH-cytochrome c reductase activity and liver weight increased by about 20 per cent; the latter increases did not represent a functionally significant induction of hexobarbital metabolism. The evidence suggests that the CBD-caused prolongation of barbiturate sleep time is the result of a decrease in the rate of barbiturate metabolism related to a decrease in cytochrome P-450 concentration. Tolerance to the effect is associated with a return to a normal metabolic rate and to normal cytochrome P-450 values.

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INTRODUCTION

Although cannabidiol (CBD) is a major constituent of marihuana [1], little is known about its pharmacological properties. Nevertheless, CBD is toxicologically important, precisely because it is a major constituent of marihuana, and also because the preclinical evaluation of its anticonvulsant properties suggests that the drug may be a useful antiepileptic agent [2]. The clinical potential of the drug is enhanced by its apparent lack of toxicity. In humans, CBD, even in massive intravenous doses, lacks the characteristic psychotoxic and cardiovascular effects of marihuana or Δ^9-tetrahydrocannabinol (Δ^9-THC) [3]. Animal studies, however, have demonstrated that CBD can prolong barbiturate sleep time [4], which was subsequently shown to be the result of the cannabinoid's ability to inhibit barbiturate metabolism by the liver [5—7]. Because CBD is an inhibitor of hepatic drug metabolism, a potential exists for toxic drug interactions with either the illicit or licit use of the drug. The following study was designed to determine the effects in mice of both acute and subacute CBD treatment on hexobarbital sleep time and on the oxidative drug-metabolizing enzymes in the liver.

MATERIALS AND METHODS

Male Charles River mice (ICR) weighing 20–30 g were used in all the experiments. CBD was suspended in isotonic saline containing 3% Tween 80, as described by Karler et al. [8], and hexobarbital was dissolved in isotonic saline immediately prior to injection. All drugs were administered intraperitoneally. The dose of CBD was 120 mg/kg which represents the anticonvulsant ED_{50} in mice in a maximal electroshock test [9].

Hepatic microsomes were prepared by a modification of the procedure of Franklin and Estabrook [10]. Livers were homogenized in 0.25 M sucrose solution (1:5, w/v), and the resulting homogenates centrifuged at 20,000 g for 20 min in a Beckman L-2 ultracentrifuge. The supernatant fraction was then centrifuged at 100,000 g for 30 min. The recovered pellet was suspended in 1.15% KCl and the suspension was centrifuged at 100,000 g for 30 min. The pellet was finally resuspended in 0.25 M sucrose solution containing 50 mM Tris-chloride buffer, pH 7.4, to obtain a final protein concentration of approximately

10 mg/ml. For the metabolic-complex detection experiments, only freshly prepared microsomes were used; for all other studies, microsomal suspensions were obtained from a frozen stock, which was stored for no longer than 4 days.

All spectral work was performed at room temperature in a Cary 118 spectrophotometer. For these studies, microsomes were suspended in 50 mM Tris-chloride buffer, pH 7.4, containing 1.15% KCl and 10 mM $MgCl_2$, to obtain a final protein concentration of 1 mg/ml. Cytochrome P-450 was measured using an extinction coefficient of 91 $mM^{-1}cm^{-1}$, as described by Omura and Sato [11].

For the spectral studies, drugs were prepared as follows: An alcoholic solution of Pluronic F68 (BASF Wyandotte Corp.) was mixed with CBD, Δ^9-THC, or piperonyl butoxide. Nitrogen was used to evaporate the ethanol; water was then added to the residue and the resulting mixture was sonicated in a Branson Sonifier W-350. The concentration of Pluronic in the drug stock preparation was 5 mg/ml and in the microsomal suspensions, 0.1 mg/ml. Drug concentrations in the microsomal suspensions were: CBD, 0.06 mM; Δ^9-THC, 0.13 mM; piperonyl butoxide, 2.4 mM. The microsome-drug suspensions were divided into the sample and reference cuvettes, and drug-metabolizing reactions were

initiated by the addition of NADPH (0.24 mM) to the sample cuvette only. During a 20-min reaction period, samples were monitored periodically for complex formation by scanning the 500—400 nm region.

Microsomes isolated from CBD-treated mice were examined for drug–cytochrome P-450 complexes formed in vivo. Ferricyanide oxidation, as described by Buening and Franklin [12], was used as a test for complexes of the SKF 525-A type. In order to detect complexes of the piperonyl butoxide type, the microsomes were divided into two cuvettes and the sample cell was reduced by the addition of a few crystals of dithionite [13].

Cytochrome c reductase activity was determined by the method of Williams and Kamin [14], protein concentrations by the method of Lowry [15], and heme determinations as described by Omura and Sato [11] and Gilbert [16]. Hexobarbital sleep times were measured from the loss to the recovery of the righting reflex, and brain hexobarbital concentrations were determined by the method of Vesell [17].

RESULTS

The time course of the prolongation of hexobarbital sleep time was determined following a single dose of CBD (Fig. 1). Significant increases in sleep times were observed for as long as 24 hr after CBD administration; and the response at 48 hr was essentially normal. In subsequent experiments, measurements of sleep time were made 4 hr after CBD treatment, because this time, associated with a marked effect, was experimentally convenient.

The effect of subacute CBD treatment on sleep time is shown in Fig. 2. Mice were given daily doses of CBD for the indicated number of days, and hexobarbital sleep times were measured 4 hr after the last dose. Animals were used only once for a sleep-time determination; therefore, each test time represented a different group of animals. The results of the repeated treatment suggest that tolerance develops to the sleep-time effect of CBD: For example, acutely administered drug prolongs sleep time about 2.5 times the control value as shown on day 1 in Fig. 2; but after 6 days of treatment, the sleep time is only slightly

Figure 1. Time course of the effect of CBD on hexobarbital sleep time. Hexobarbital (100 mg/kg) sleep times were measured 1, 4, 12, 24 and 48 hr after a single dose of CBD (120 mg/kg). Each value is expressed as the mean and standard deviation of a group of 20 animals, and no animals were used more than once. The asterisks (*) indicate the values that are significantly different from their corresponding controls, as determined by a t test ($P < 0.05$).

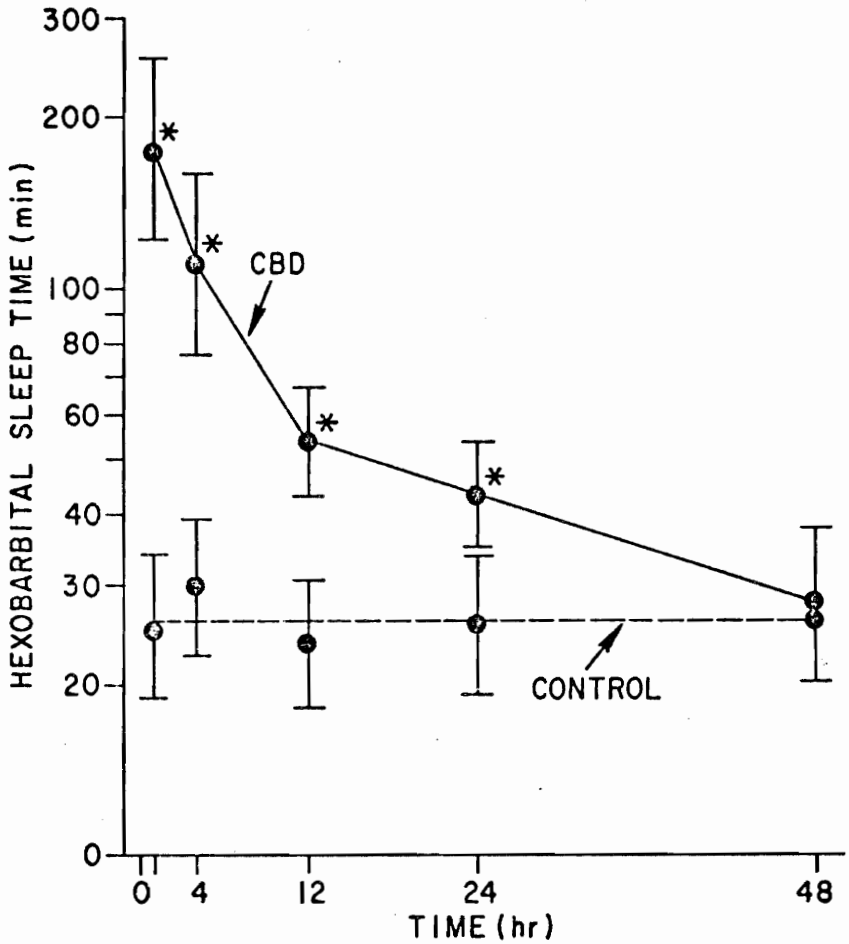

Figure 1. Time course of the effect of CBD on hexobarbital sleep time

Figure 2. Effect of subacute CBD treatment on hexobarbital sleep time. Animals were treated every 24 hr with CBD (120 mg/kg) for the indicated number of days. Hexobarbital (100 mg/kg) sleep times were measured 4 hr after the final treatment; each treatment group consisted of 13–17 animals and the mean and standard deviation for each group are shown. The data were subjected to a regression analysis; the slope of the line is −0.058 and the correlation coefficient is 0.63. The slope is significantly different from zero, as determined by a t test ($P < 0.01$).

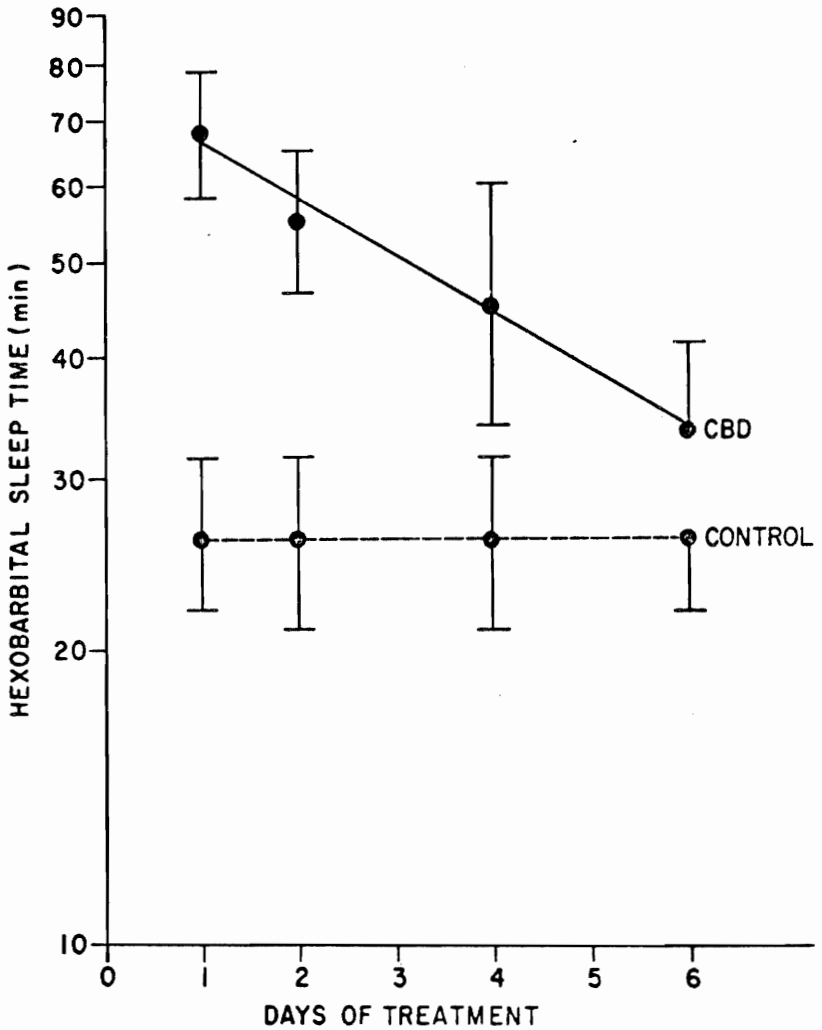

Figure 2. Effect of subacute CBD treatment on hexobarbital sleep time

longer than that of the control. Furthermore, an analysis of the data indicates that the slope of the line in Fig. 2 is negative and significantly different from zero ($P < 0.01$), thus confirming the interpretation that tolerance rapidly develops to the effect of CBD on hexobarbital sleep time. In subsequent studies of tolerance, animals were given CBD daily for 4 days, because this treatment period was adequate to produce a measurable degree of tolerance.

The data in Table 1 were obtained from experiments that were designed, in part, to find out whether the CBD-enhanced sleep time was the result of an increased sensitivity of the central nervous system (CNS) to hexobarbital. The results in Table 1 show that 4-hr pretreatment with CBD increased sleep time from 39 min to 111 min; however, the brain concentration of hexobarbital upon awakening was unchanged, which suggests that the drug, acutely administered, did not interact centrally with the barbiturate. The subacutely treated group in this study clearly showed the development of tolerance to CBD, as evidenced by the decrease in sleep time from 111 min in the acutely treated to 62 min; the tolerance, however, was not accompanied by a decrease in the sensitivity of the CNS to hexobarbital, because, upon awakening, the brain concentration of the barbiturate in the subacutely treated mice (38 μg/g brain) was not significantly different from that in

Table 1. Effect of acute and subacute CBD treatment on the awakening brain concentration of hexobarbital

	Hexobarbital	
Treatment*	Sleep Time† (min)	Brain Concentration‡ (μg/kg
Control	39 ± 7*	37 ± 6
Acute	111 ± 14*	35 ± 8
Subacute	62 ± 20*	38 ± 7

* Control animals received vehicle for 4 days; acute, vehicle for 3 days and CBD (120 mg/kg) on the fourth day; subacute, CBD (120 mg/kg) for 4 days. All measurements were made 4 hr after the last treatment.

† Sleep times were measured following a single i.p. dose of hexobarbital (100 mg/kg). Each value represents a mean and standard deviation of 15 animals, and all values are significantly different from each other, as determined by the multiple range test ($P < 0.05$).

‡ Brain hexobarbital concentration upon awakening was determined following a single dose of hexobarbital (100 mg/kg). Each value represents a mean and standard deviation of 12 animals. There are no significant differences between any of the values as determined by the multiple range test ($P < 0.05$).

the acutely treated group (35 μg/g brain).

The results shown in Fig. 3 illustrate that acute CBD treatment increases the half-time of the barbiturate in the brain from a control value of 22 min to 53 min; such an effect is indirect evidence that the rate of hexobarbital metabolism is decreased by CBD. In the subacutely treated animals the half-time is 33 min, which is a significant reduction from the 53-min half-time in the acutely treated group; that is, the barbiturate half-time tends to return to normal in tolerant animals.

The above evidence indicates that a likely site of action of CBD is the hepatic microsomal drug-metabolizing system; consequently, the drug's effect on cytochrome P-450 concentration was investigated (Fig. 4). A single dose of CBD caused a significant decline in cytochrome P-450 concentration, an effect which lasted for 12 hr after drug administration; the duration of this decline approximates that of the effect on sleep time (Fig. 1). Such depression in cytochrome P-450 concentration, however, was not followed in the recovery period by a greater-than-normal cytochrome P-450 concentration; that is, no rebound phenomenon was recorded either at 24 or 48 hr after treatment, suggesting that the rapid development of tolerance does not appear to involve cytochrome P-450 induction.

Figure 3. The half-times of hexobarbital in the brain following acute and subacute treatment with CBD. The acute group received vehicle daily for 3 days, CBD the fourth day; the subacute, CBD daily for 4 days; and the control, vehicle daily for 4 days. The dose of CBD was 120 mg/kg. Four hr after the final treatment, each animal received a single dose of hexobarbital (130 mg/kg). Each point represents the mean brain-hexobarbital concentrations of 5 animals. The slopes of the lines, determined by linear regression analysis, are −0.013 for the acute, −0.022 for the subacute, and −0.031 for the control treatment; the correlation coefficients are in parentheses. The slopes are significantly different from each other, as determined by a t test ($P < 0.05$). The half-times of hexobarbital in the brain were calculated from the first-order decay equation.

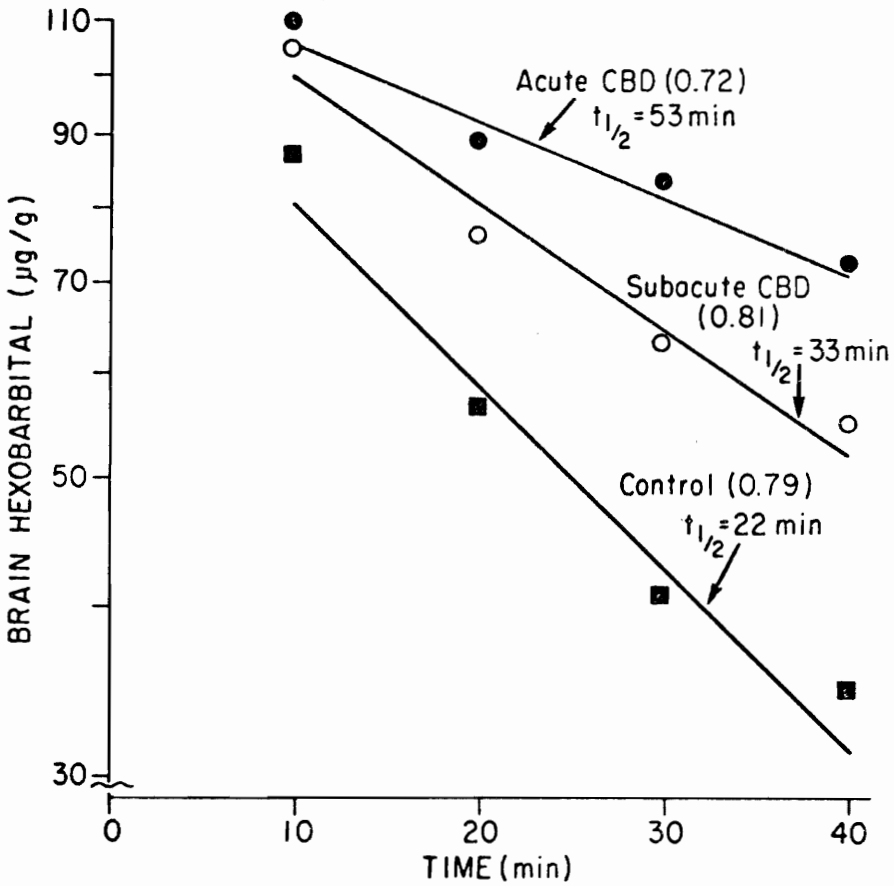

Figure 3. The half-times of hexobarbital in the brain following acute and subacute treatment with CBD

Figure 4. Time course of the effect of CBD on cytochrome P-450 concentration. Each animal was given a single dose of CBD (120 mg/kg) or vehicle and the concentration of cytochrome P-450 was determined 1, 4, 12, 24 and 48 hr after drug administration. The results are expressed as a percentage of control. The values represent means of groups consisting of 9–13 animals; the ranges are in parentheses. The mean absolute concentration of cytochrome P-450 in the controls was 0.72 nmoles/mg microsomal protein. The asterisks (*) indicate values that are significantly different from their corresponding controls, as determined by the Mann-Whitney U test ($P < 0.05$).

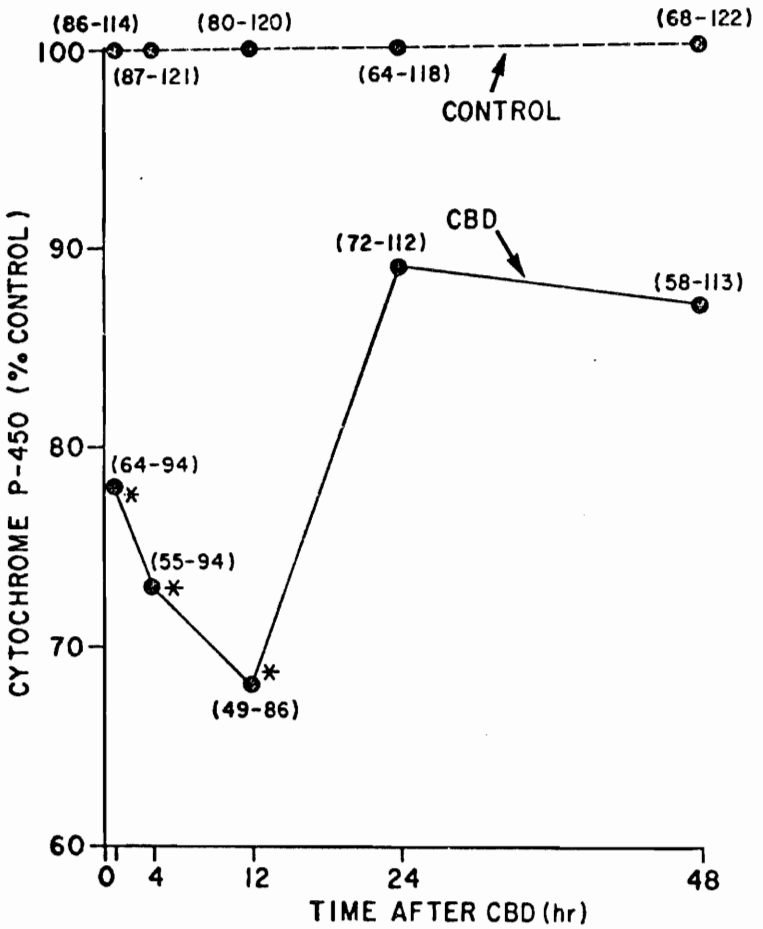

Figure 4. Time course of the effect of CBD on cytochrome P-450 concentration

The in-vitro microsomal metabolism of CBD was studied in order to determine whether the decline in cytochrome P-450 was due to a CBD metabolite—cytochrome P-450 complex. In these studies, CBD was compared to Δ^9-THC (a cannabinoid which is not a potent inhibitor of drug metabolism [5—7]) and to piperonyl butoxide (a compound which is known to produce metabolic—intermediate complexes with cytochrome P-450 [13]). CBD, Δ^9-THC and piperonyl butoxide were metabolized in the presence of hepatic microsomes and NADPH, and each system was monitored spectrophotometrically (Fig. 5). Two concentrations of CBD and Δ^9-THC were used in these experiments: one approximately equal to the K_m, and the other, 10 times the K_m for the metabolism of the drugs. The spectral data from only the higher drug-concentration experiments are shown in Fig. 5, but the results from the lower drug concentrations were identical. Under these conditions, piperonyl butoxide produced its characteristic metabolic—intermediate complex absorption spectrum with a maximum at 455 nm; however, neither CBD nor Δ^9-THC formed a spectrophotometrically detectable complex (Fig. 5A).

The dithionite reduction of the microsomes following in-vitro metabolism resulted in the appearance of an absorption

Figure 5. Spectral analyses of microsomal systems metabolizing CBD, Δ^9-THC and piperonyl butoxide.

A. Analysis for metabolic-intermediate complexes with cytochrome P-450. Hepatic microsomes were prepared from an untreated mouse; microsomal suspensions contained 1 mg protein/ml. The concentrations of CBD (0.06 mM) and Δ^9-THC (0.13 nM) were approximately 10 times the apparent K_m values for these drugs. The concentration of piperonyl butoxide was 2.4 mM. NADPH (0.24 mM) was added only to the sample cuvette. After a reaction time of 9 min at room temperature, a difference spectrum was obtained (500-400 nm).

B. Effect of dithionite reduction on the spectral analysis of the reaction systems in 5A. After a reaction time of 10 min, microsomal systems in A were reduced by the addition of dithionite and a difference spectrum was obtained.

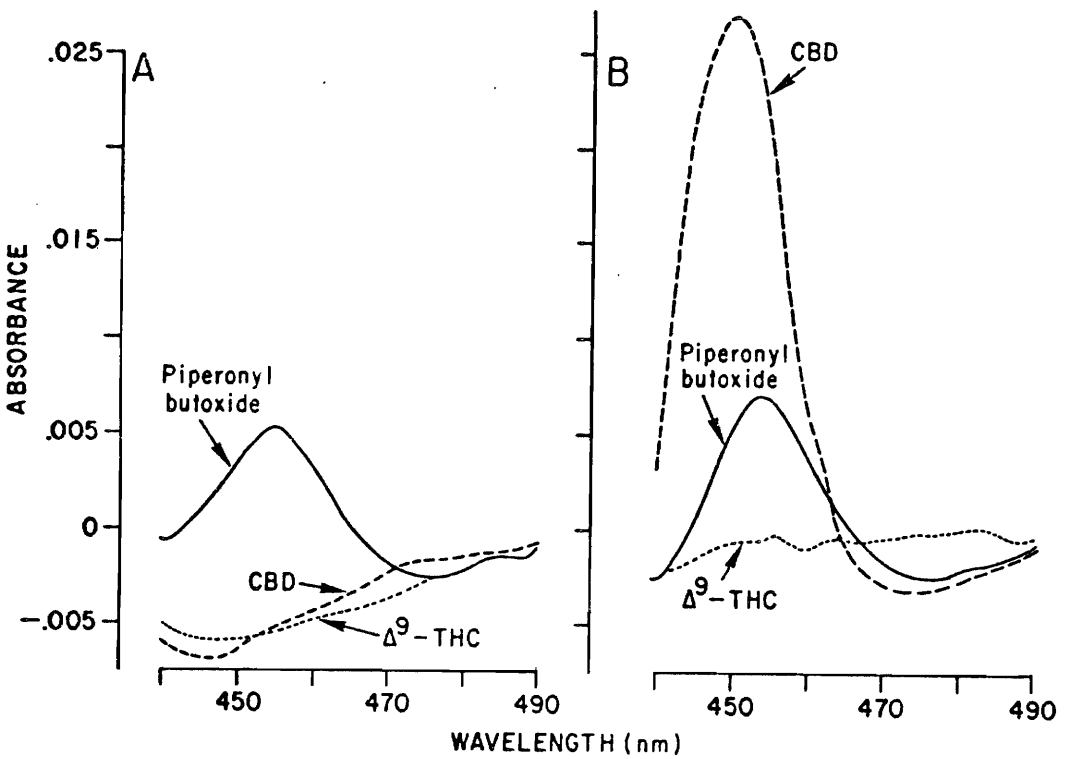

Figure 5. Spectral analyses of microsomal systems metabolizing CBD, Δ^9-THC and piperonyl butoxide

spectrum with a maximum at 450 nm (Fig. 5B). This result was uniquely associated with the CBD system and not with either Δ^9-THC or piperonyl butoxide. The appearance of cytochrome P-450 suggested that CBD metabolism endogenously generated carbon monoxide; and, in fact, the complex was dissociated by bubbling the cuvette with oxygen. The apparent generation of carbon monoxide was also shown to require CBD metabolism; that is, a non-metabolizing system containing only CBD and microsomes did not cause the appearance of the cytochrome P-450 absorbance. Moreover, the data indicate that the source of the carbon monoxide is not the enzymic destruction of cytochrome P-450, because the concentration of P-450 did not change during the in-vitro metabolism of CBD; both initial and final concentrations of cytochrome P-450 (before and after 10-min incubation) were 0.6 nmoles/mg protein.

Fig. 4 shows that in acutely treated animals CBD caused a decrease in cytochrome P-450; in order to determine if such decrease in vivo was due to a reduction in the available cytochrome P-450 by the formation of a metabolite complex, the following experiment was performed: Animals were given a single dose of CBD and their livers were removed 4 hr later, at which time a significant in-vivo depression in the concentration of cytochrome

P-450 was recorded (Fig. 4). Microsomes prepared from these livers were divided into two cuvettes and one cell was either oxidized with ferricyanide or reduced with dithionite. The cells were spectrophotometrically scanned in the 500—400 range, and no complex formation was revealed. The observed decrease in cytochrome P-450 cannot, therefore, be attributed to the presence of complexes of the SKF 525-A and piperonyl butoxide types.

An attempt was also made to discover whether the decline in cytochrome P-450 was reflected by a decrease in total microsomal heme content: Total heme was measured in 12 control and 12 CBD-treated animals 12 hr after drug administration. The mean and standard deviation of the control group was 1.2 ± 0.17, compared with 1.1 ± 0.14 nmoles/mg microsomal protein for the drug-treated group. These values do not differ statistically; similar results were obtained 1 hr after drug administration.

The data on the half-time of brain hexobarbital in Fig. 3 suggest that the tolerance observed in subacutely treated animals involves either a reversal of the inhibitory effect, or a compensatory increase in the activity (induction) of the microsomal enzyme system. In order to test the latter possibility, a determination of the influence of acute and subacute CBD treatment on some hepatic constituents was essayed (Table 2). In these experiments, mice

Table 2. Effect of acute and subacute CBD treatment on the liver*

	Control	% Control	
		Acute	Subacute
Liver weight †	100 (84-113)	--	121‡ (107-139)
Liver protein/g liver †	100 (93-105)	--	102 (86-115)
Microsomal protein/ g liver †	100 (78-119)	--	113 (91-146)
Cytochrome P-450/g microsomal protein ‡	100 (77-137)	78‡ (57-86)	99 (81-135)
Cytochrome c reductase/ g microsomal protein ‡	100 (85-112	99 (85-114)	117‡ (98-157)

* Control group received vehicle daily for 4 days; acute, vehicle daily for 3 days and CBD on the fourth day; subacute, CBD daily for 4 days. Each value is expressed as a percentage of the control and represents a mean of 8–12 animals; the range of each value is in parentheses. The absolute mean values for the control group are: liver, 1.7 g; liver protein, 263 mg/g liver; microsomal protein, 13 mg/g liver; cytochrome P-450, 0.77 nmoles/mg microsomal protein; and cytochrome c reductase activity, 77 nmoles/min/mg microsomal protein.

† Determined 24 hr after the last treatment.

‡ Value is significantly different from control, as determined by the Mann-Whitney U test ($P < 0.05$).

‡ Determined 4 hr after the last treatment.

were treated with CBD once (acute) or daily for 4 days (subacute). Twenty-four hr after the last dose of CBD, liver weight increased significantly by 21 per cent; however, the concentrations of liver protein and microsomal protein were unaffected, which suggests that the increase in liver weight is a consequence of an increase in the number of hepatic cells and not a change in the composition of the existing cells. The hepatic microsomal content of cytochrome P-450 and cytochrome c reductase was measured in another group of animals 4 hr after the final dose of CBD: Acute CBD treatment produced a significant 22 per-cent decline in cytochrome P-450 concentration, but in the subacute group there was no effect on the cytochrome, pointing to a development of tolerance. Cytochrome c reductase results were the reverse of those for cytochrome P-450; that is, in acutely treated animals there was no change in the activity of the reductase; however, in the subacutely treated group the activity was elevated significantly by 17 per cent.

The above measurements in tolerant animals revealed only two possible signs of microsomal induction: an increase in liver weight and in cytochrome c reductase activity. The functional significance of these changes in terms of hexobarbital sleep time was measured in tolerant animals. The data shown in Table 3 were obtained from animals treated daily with CBD for the

Table 3. Influence of daily CBD administration on hexobarbital sleep time

Days of Treatment	Sleep Time*	
	Vehicle	CBD
1	26 ± 8	44 ± 10†
2	20 ± 5	31 ± 10
4	19 ± 7	20 ± 6
8	21 ± 6	30 ± 8
12	21 ± 5	32 ± 7
12‡	29 ± 9	32 ± 6

* Hexobarbital (100 mg/kg i. p.) sleep times in groups 1 through 12 were measured 24 hr after the final CBD treatment (120 mg/kg/day). All sleep-time values represent means and their standard deviations of groups of 20 mice. Each group was tested for sleep time once only.

† Value is significantly different from control as determined by a t test ($P < 0.05$).

‡ Sleep time assessed 48 hr after final CBD treatment.

indicated number of days, and hexobarbital sleep times were determined either at 24 or 48 hr after the last dose of CBD. For as long as 12 days of daily CBD treatment, there was no reduction in hexobarbital sleep time; therefore, the CBD tolerance cannot be ascribed to a functionally significant induction of the hexobarbital-metabolizing enzymes.

DISCUSSION

The ability of CBD to inhibit hepatic drug metabolism poses a toxic potential in terms of drug interactions, whether the drug is used for licit or illicit purposes. The dose of CBD in illicit usage is several orders of magnitude lower than that of the present study, and the work of Dalton et al. [18] suggests that the relatively low doses derived from marihuana do not affect secobarbital metabolism in humans. Because of the interest in CBD as an antiepileptic agent, the principal goal of the present study was to determine and define some of the characteristics of the interaction between anticonvulsant doses of CBD and the hepatic microsomal drug-metabolizing enzymes. The dose of CBD selected for the investigation, 120 mg/kg, is the anticonvulsant ED_{50} for mice in a maximal electroshock test [9]. The in-vivo drug-metabolzing activity was assessed in terms of hexobarbital sleep time because hexobarbital is primarily metabolized by the hepatic microsomal enzymes [19] and the sleep time is proportional to the activity of these enzymes [17].

Acutely administered CBD was shown to prolong

significantly hexobarbital sleep time in mice for 24 hr, an effect similar to that described by Paton and Pertwee [5]. There appear, however, to be species differences in the duration of CBD's sleep-time effect: For example, Siemens et al. [7] reported that cannabis extracts enhanced pentobarbital sleep time in the rat for as long as 63 hr after drug administration. Regardless of these species differences, the mechanism of the inhibiton appears to be the same. Previous studies with lower CBD doses (10 mg/kg) demonstrated that the prolongation of barbiturate sleep time is not the result of a central interaction between CBD and the barbiturate, but the consequence of CBD's ability to inhibit barbiturate metabolism by the liver [4–6]. These conclusions were also found to be valid for the relatively high doses of CBD used in the present study. The concentration of hexobarbital in the brain upon awakening was unchanged following acute CBD treatment, indicating that the drug did not alter the sensitivity of the CNS to the barbiturate. Furthermore, the treatment increased the half-time of hexobarbital in the brain, indirect evidence that CBD decreased the rate of hexobarbital metabolism.

In the subacute experiments, tolerance rapidly developed to the CBD effect on hexobarbital sleep time, and associated with the tolerance was a return of the hexobarbital half-time in brain

toward the control values. The decrease in hexobarbital half-time may result from an increased rate of drug metabolism, and the possibility that tolerance is of the dispositional, rather than the cellular, type is further bolstered by the failure of subacute CBD treatment to change the brain concentration of hexobarbital upon awakening; in other words, the tolerance was not related to an alteration in the sensitivity of the CNS to the barbiturate.

The above findings imply that both the acute and subacute effects of CBD involve the hepatic drug-metabolizing system. An investigation of the effect of CBD on this system revealed that a single dose of CBD caused a significant depression in cytochrome P-450 concentration, an observation of particular interest because the cytochrome has been implicated as the site of action of two other inhibitors of drug metabolism, SKF 525-A and piperonyl butoxide; that is, both of these compounds are believed to inhibit drug metabolism by inactivating cytochrome P-450 [12, 13]. In addition, the magnitude and duration of the CBD-induced decline in cytochrome P-450 concentration was similar to that produced by SKF 525-A.

The decrease in the concentration of cytochrome P-450 may account for CBD's inhibitory effect on hexobarbital metabolism. This conclusion is supported by the evidence that the

duration of the cytochrome P-450 decline following acute CBD treatment approximates the duration of the sleep-time prolongation, about 24 hr. Furthermore, following subacute CBD treatment, the cytochrome P-450 concentration is restored to the normal value; therefore, animals that are tolerant to the effect of CBD on sleep time are also tolerant to the drug's action on cytochrome P-450.

Levin et al. [20] reported that the degradation of cytochrome P-450 in the rat is a biphasic process, with a half-time of 7—8 hr for the fast and 42—46 hr for the slow phase. CBD's depression of cytochrome P-450, which has a rapid onset, could possibly be the result of inhibition of the synthesis of the cytochrome; however, the above suggestion is only plausible if most of the cytochrome P-450 in the mouse were very rapidly degraded. Since the degradation rate in the mouse is not known, two other possible mechanisms of the decline in cytochrome P-450 were considered: Either CBD could promote the destruction of the cytochrome or it could interfere with its spectral detection. The metabolism of CBD was studied in order to distinguish between these two alternatives.

SKF 525-A and piperonyl butoxide form metabolic-intermediate complexes with cytochrome P-450 which prevent carbon-

monoxide binding and, thereby, reduce the amount of spectrally measurable cytochrome P-450 [12, 13]. The complexes themselves have characteristic absorption maxima that can be detected in microsomes following both in-vivo and in-vitro metabolism of these drugs. In contrast, the in-vivo administration of CBD did not yield a spectrally detectable complex, although there was a significant in-vivo decline in cytochrome P-450 at the time of the measurement. Moreover, no complex absorption maxima and no decline in spectrally measurable cytochrome P-450 were seen after the in-vitro metabolism of either relatively low (K_m) or high (10 times K_m) concentrations of CBD, even though the high concentration (0.06 mM) was probably sufficient to inhibit non-competitively hexobarbital metabolism in vitro [6].

Because the recorded in-vivo decrease in cytochrome P-450 cannot be accounted for by complex formation, the decrease may be a consequence of a destruction of cytochrome P-450. Such an effect has been reported for many other drugs, including allylisopropylacetamide and secobarbital [21, 22]. A change in the absolute amount of cytochrome P-450 could be determined by quantitating total heme. Such an assessment, however, failed to detect any significant decrease in total microsomal heme concentration.

As described earlier, repeated CBD treatment is accompanied by a restoration of the normal rate of hexobarbital metabolism, which is indicative of a development of tolerance. Such tolerance may be due either to a reversal of the inhibitory action of CBD or to a compensatory increase in the activity of the microsomal enzyme system. For example, SKF 525-A has been shown to induce the activity of the hepatic microsomal system [23, 24], and Buening and Franklin [12] demonstrated that repetitive treatment produced a marked increase in the concentration of cytochrome P-450. In order to determine whether CBD tolerance is the result of induction, the influence of subacute CBD on some constituents of the drug-metabolizing system was assessed. Unlike SKF 525-A, subacute CBD treatment does not increase the cytochrome P-450 concentration; in addition, microsomal protein per g liver and liver protein per g liver remained unchanged. The only signs of induction were an increase in cytochrome c reductase activity and in total liver weight. Following CBD withdrawal after 12 days of daily treatment, however, sleep time was normal; therefore, CBD tolerance cannot be ascribed to a functionally significant induction of the hexobarbital-metabolizing enzymes.

Generally speaking, the results of the present study are

consistent with other investigators' findings that CBD prolongs sleep time by inhibiting the hepatic drug-metabolizing system. Although the precise mechanism of CBD's inhibitory action is still unknown, it does appear that the depression of cytochrome P-450 may account, at least in part, for CBD's effect on drug metabolism. Finally, the rapid development of tolerance to the inhibitory effect of CBD may serve to minimize potentially toxic drug interactions arising from chronic exposure to the drug.

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